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14. ABSTRACT

Biosensors suitable for providing informed rapid treatment analysis for soldiers and real-time environmental monitoring of toxic agents were developed. In particular, glass encapsulated field-effect sensors that are chemically robust and batch-fabricated by a conventional 6" wafer process were developed and demonstrated for the following applications: i) Polymerase Chain Reaction (PCR) product quantification, ii) Integrated microelectronic device for label-free nucleic acid amplification and detection, and iii) Monitoring of heparin and its low molecular weight analogs.

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Development of microdevices for biomolecular detection
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February 1, 2004 to December 31, 2007

FINAL REPORT for the
Air Force Office of Scientific Research (AFOSR)

June 2008

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Status of effort

Glass encapsulated field-effect biosensors that are chemically robust and batch-fabricated by a conventional 6" wafer process were developed and demonstrated for the following applications:

i) Polymerase Chain Reaction (PCR) product quantification. Field-effect sensor can sequentially detect the intrinsic charge of multiple unprocessed PCR products. The product concentration can be monitored at various stages of PCR and a readout can be generated that resembles that of a real-time fluorescent measurement using an intercalating dye but without its potential inhibition artifacts.

ii) Integrated microelectronic device for label-free nucleic acid amplification and detection. By combining amplification and detection on the same device, we show that the presence or absence of a particular DNA sequence can be determined by converting the analog surface potential output of the field-effect sensor to a simple digital true/false readout.

iii) Monitoring of heparin and its low molecular weight analogs. Monitoring and control of the heparin level in a patient's blood during and after surgery is essential, but current clinical methods are limited to indirect and off-line assays. We have been able to detect heparin and heparin-based drugs with high sensitivity and selectivity.

These devices can ultimately be configured to provide informed rapid treatment analysis for soldiers and real-time environmental monitoring of toxic agents.

Accomplishments/New Findings

Polymerase Chain Reaction (PCR) product quantification

The introduction of real-time monitoring of the polymerase chain reaction (PCR) represents a major breakthrough in specific nucleic acid quantification. This technique employs fluorescent intercalating agents or sequence-specific reporter probes to measure the concentration of amplified products after each PCR cycle. However, the need for optical components can limit the scalability and robustness of the measurement for miniaturization and field-uses. Moreover, the addition of external fluorescent reagents can induce inhibitory effects and require extensive optimization.

We have developed a robust and simple method for direct label-free PCR product quantification using an integrated microelectronic sensor (Figure 1). The field-effect sensor can sequentially detect the intrinsic charge of multiple unprocessed PCR products and does not require sample processing or additional reagents in the PCR mixture. The sensor measures nucleic acid concentration in the PCR relevant range and specifically detects the PCR products over reagents such as Taq polymerase and nucleotide monomers. The sensor can monitor the product concentration at various stages of PCR and can generate a readout that resembles that of a real-time fluorescent measurement using an intercalating dye but without its potential inhibition artifacts (Figure 2). The device is mass-produced using standard semiconductor processes, can be reused for months, and integrates all sensing components directly on-chip. As such, our approach establishes a foundation for the direct integration of PCR-based in vitro biotechnologies with microelectronics.

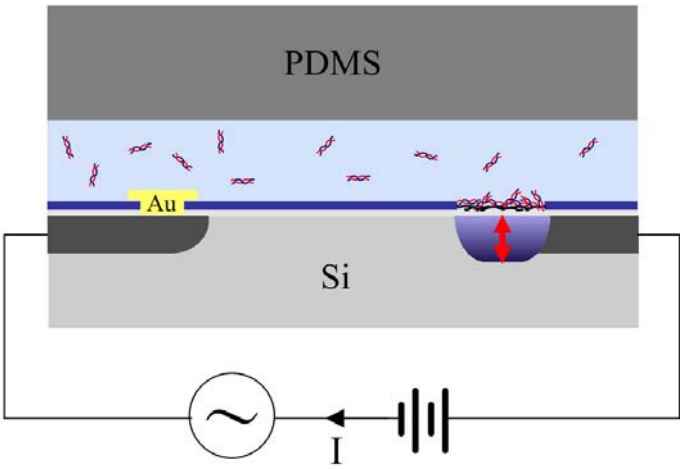

Figure 1: Cross-sectional drawing demonstrating the basis of the device measurement. Binding of charged molecules such as DNA on the sensor's surface alters the distribution of positive mobile charge carrier in silicon, results in a modulation of the depletion depth (red arrow), hence changing the capacitance. This change in capacitance is monitored by measuring the ac current between the sensor and the gold electrode.

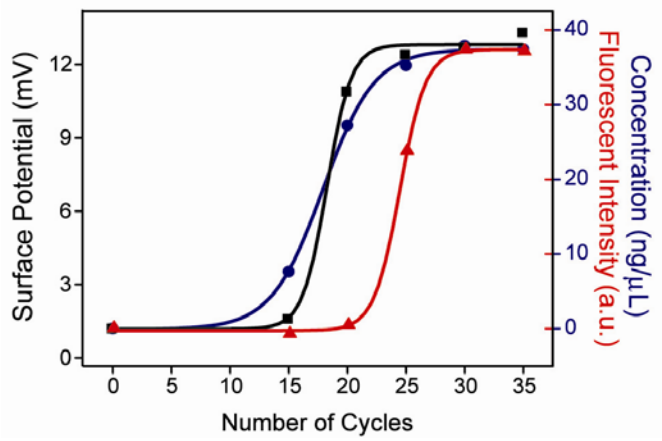

Figure 2: Comparison between steady state response of electronic measurements (black), real-time monitoring of PCR using Sybr Green I intercalating dye (red), and concentration analysis of the products using DNA Labchip kits (blue). No fluorescent labels were used for electronic detection and concentration measurements. However the discrepancy with the Sybr Green I measurement is likely due to partial inhibition of the PCR reaction by the fluorescent reagent.

Integrated microelectronic device for label-free nucleic acid amplification and detection

While there have been extensive advances in miniaturized polymerase chain reaction (PCR) systems, progress on integrated microfabricated readout mechanisms have been rather limited, and most systems rely on off-chip optical detection modules to measure the final product. Existing optical detection platforms typically include CCD cameras, photodiodes, and photomultiplier tubes. While such hardware has adequate sensitivity for detecting PCR product in sample volumes significantly lower than that of bench-top systems, most are difficult to miniaturize and integrate into a compact analytical system. For example, some portable systems incorporating external LEDs and photodetectors can weigh between 1kg and 4kg each. To address these limitations, several groups have successfully embedded photodetectors within an integrated PCR platforms. However, these devices still rely on external excitation sources.

To address this limitation, we have developed an integrated microelectronic device for amplification and label-free detection of nucleic acids (Figure 3). Amplification by PCR is achieved with on-chip metal resistive heaters, temperature sensors, and microfluidic valves. We demonstrate a rapid thermocycling with rates of up to 50 °C/s and a PCR product yield equivalent to that of a bench-top system. Amplicons within the PCR product are detected by their intrinsic charge with a silicon field-effect sensor. Similar to existing optical approaches with intercalators such as SYBR Green, our sensing approach can directly detect standard double-stranded PCR product, while in contrast, our sensor occupies a micron-scale footprint, dissipates only nano-watt power during operation, and does not require labeling reagents. By combining amplification and detection on the same device, we show that the presence or absence of a particular DNA sequence can be determined by converting the analog surface potential output of the field-effect sensor to a simple digital true/false readout (Figure 4).

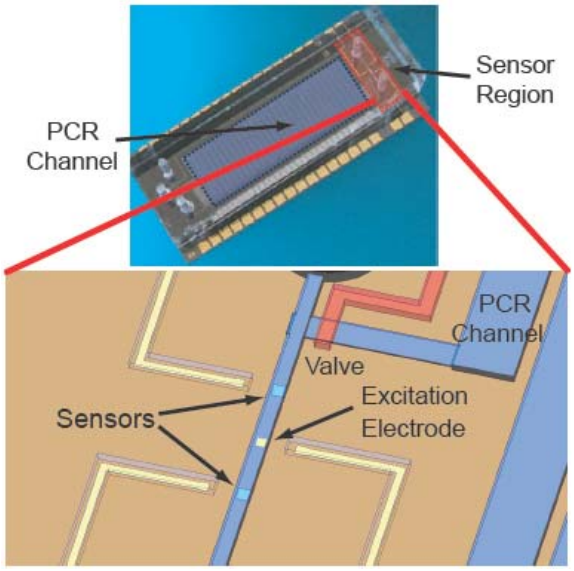

Figure 3: Device layout and concept. (top) Photograph of an integrated device with embedded sensors (right dotted area), PCR microfluidic channel with integrated valves (left dotted area), and metal resistive heaters and temperature sensors (features above and below PCR channel). (bottom) 3D rendering of device centered on sensors (top and bottom squares) and excitation metal electrode. Adjacent features include gold traces for electrical connections, inlet of sensor channel, and an integrated valve controlling the interface to the PCR channel.

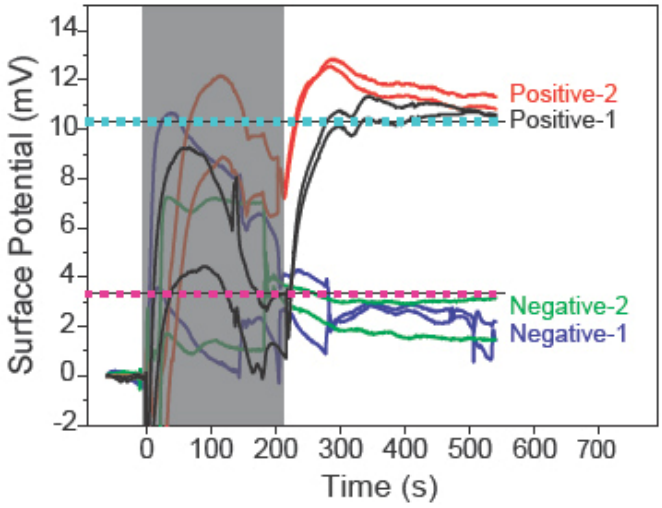

Figure 4: Integrated PCR and field-effect sensing of product. The sensor was functionalized, pressure was applied to flow the content of PCR channel over electronic sensors, after which valves were closed and measurement buffer flow was restored. The PCR channel was replenished with starting PCR reagent as its content was flowed into the sensing channel. The grey-out area indicated the period of PCR channel injection during which mechanical operations caused the sensor to lose its baseline value and drift temporarily. The higher and lower dotted segments are arbitrarily defined threshold levels for positive and negative signals, respectively.

Monitoring of heparin and its low molecular weight analogs

Heparin is a highly sulfated glycosaminoglycan that is used as an important clinical anticoagulant. Monitoring and control of the heparin level in a patient's blood during and after surgery is essential, but current clinical methods are limited to indirect and off-line assays. We have developed a silicon field-effect sensor for direct detection of heparin by its intrinsic negative charge. The sensor consists of a simple microfabricated electrolyte-insulator-silicon (EIS) structure encapsulated within microfluidic channels (Figure 5). As heparin-specific surface probes we used the clinical heparin antagonist protamine or the physiological partner antithrombin III. The dose-response curves in 10% PBS revealed a detection limit of 0.001 U/ml which is orders of magnitude lower than clinically relevant concentrations. We also detected heparin-based drugs such as the low molecular weight heparin enoxaparin (Lovenox®) and the synthetic pentasaccharide heparin analog fondaparinux (Arixtra®) (Figure 6) which cannot be monitored by the existing near-patient clinical methods. We demonstrated the specificity of the antithrombin III functionalized sensor for the physiologically active pentasaccharide sequence. As a validation, we showed correlation of our measurements to those from a colorimetric assay for heparin-mediated anti-Xa activity. These results demonstrate that silicon field-effect sensors could be used as a portable device for routine monitoring and maintenance of therapeutic levels of heparin and heparin-based drugs.

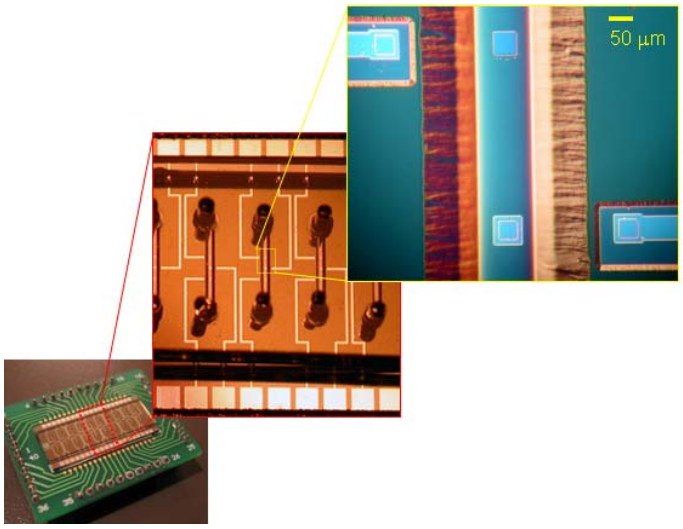

Figure 5: Optical micrograph of an array of silicon field-effect sensors for the detection of charged biomolecules such as heparin.

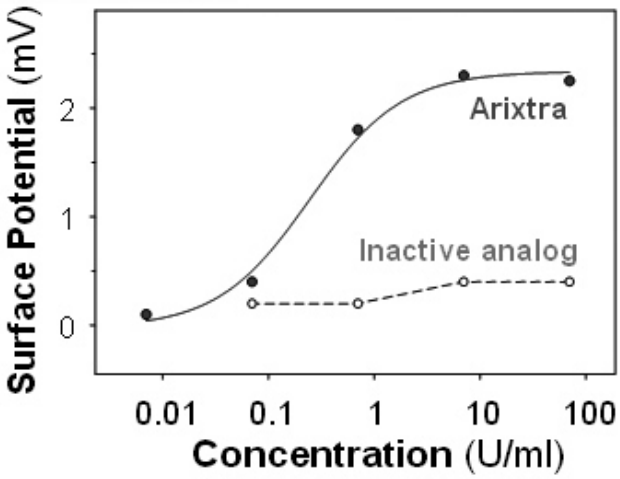

Figure 6: Dose-response curve of the AT-III-sensor for heparin-based pentasaccharide drug fondaparinux (●) and 6-O desulfated fondaparinux (○), which is known to exhibit low binding affinity for AT-III. Data points for fondaparinux are fit with a Langmuir isotherm (solid line), and those for and 6-O desulfated fondaparinux are connected with a dashed line. Note that 0.2 U/mL ~ 1mg/ml ~ 50 nM

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Publications

N. Milovic, J. Behr, M. Godin, C.J. Hou, K.R. Payer, A. Chandrasekaran, P.R. Russo, R. Sasisekharan, S.R. Manalis. Monitoring of Heparin and Its Low Molecular Weight Analogs by Silicon Field Effect. Proceedings of the National Academy of Sciences, 103 13374 (2006).

J. Hou, M. Godin, K. Payer, R. Chakrabarti, S.R. Manalis. Integrated Microelectronic Device for Label-free Nucleic Acid Amplification and Detection. Lab on a Chip, 7 347 (2007).

C.J. Hou, N. Milovic, M. Godin, P.R. Russo, R. Chakrabarti, S.R. Manalis. Label-free Microelectronic PCR Quantification. Analytical Chemistry, 78 2526 (2006).

Interactions/Transitions:
Participation/presentations at meetings, conferences, seminars, etc.
10th International Conference on Miniaturized Systems for Chemistry and Life Sciences (μTAS2006)
IEEE Sensors
APS
MRS
National Academy of Engineering (NAE)
BioDetection
Cornell, seminar
Harvard, seminar
Hewlett Packard Corvallis, seminar

Consultative and advisory functions to other laboratories and agencies
None

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Field-effect sensors developed under this program were evaluated by Hewlett Packard Corvallis

Patent disclosures
Method and apparatus for label-free electronic real-time double-stranded nucleic acid detection (20080124717)
Monitoring heparin by microelectronic devices (20070212786)

Honors/Awards
None.